IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Keith D. Hendricks
Group Art Unit	:	1761
Applicant	:	David Vincent Zyzak et al.
Application No.	:	10/606,137
Confirmation No.	:	3971
P&G Docket No.	:	9043MXL
Filed	:	June 25, 2003
For	:	METHODS FOR REDUCING ACRYLAMIDE IN FOODS, FOODS HAVING REDUCED LEVELS OF ACRYLAMIDE, AND ARTICLE OF COMMERCE

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED PURSUANT TO 37 C.F.R. § 1.131

Sir:

I, David Vincent Zyzak, declare that:

1. I am a Senior Scientist at The Procter & Gamble Company ("P&G"), Winton Hill Business Center, 6300 Center Hill Avenue, Cincinnati, Ohio, 45224.

2. I understand that myself, Robert Alan Sanders, Marko Stojanovic, David Cammiade Gruber, Peter Yau Tak Lin, Maria Dolores Martinez-Serna Villagran, John Keeney Howie and Richard Gerard Schafermeyer ("Zyzak") are the named inventors of U.S. patent application Serial No. 10/606,137 (the "Zyzak '137 application"). I make this declaration in support of Zyzak's claim that the invention claimed in the Zyzak '137 application was made before the September 19, 2002 priority date of Elder et

al.'s U.S. patent application 10/247,504 (the "Elder '504 application"), as well as before the September 11, 2002 publication of the Health Canada Letter entitled "Acrylamide in Food Update" (the "Health Canada Letter"). The Health Canada Letter was submitted to the US PTO in an IDS filed on June 14, 2006. I also understand that Zyzak is requesting that the U.S. Patent and Trademark Office declare an interference between the Zyzak '137 application, and the Elder '504 application.

3. I received a B.S. in Chemistry from Old Dominion University in 1989. I received a Ph.D. in Biochemistry from the University of South Carolina in 1995. My Ph.D. thesis was "Studies on the Maillard reaction: mechanism of the fructosamine assay, decomposition of Amadori adducts on protein, and reaction of 3-deoxyglucosone with arginine residues in protein."

4. Since I earned my Ph.D. in 1995, I have continuously been employed in research and development positions in the food industry. I am the author of numerous publications related to my research and development work in the food industry.

5. From August 1995 until November 1997, I worked for Nestle in New Milford, Connecticut, as a Developmental Technologist and Process Flavor Chemist.

6. From November 1997 until September 1999, I worked for Takasago Institute, a flavors and fragrances company located in Rockleigh, New Jersey. My position at Takasago was Senior Scientist.

7. In September 1999, I started working for P&G in Cincinnati, Ohio. When I joined P&G, my position was Scientist in P&G's Food and Beverage Analytical/Microbiology Division. In September 2000, I was promoted to Senior Scientist. In 2002, the name of the Food and Beverage Analytical/Microbiology Division was changed to Snacks and Beverage Analytical/Microbiology. In 2004, the name was changed again to Household Care Analytical. Today I am a Senior Scientist in P&G's Household Care Analytical Division. I am also the Coordinator of Coffee Analytical Support. During my employment at P&G, I have worked continuously in research and development related to snack food products.

8. I conducted an experiment entitled "Use of Asparaginase to decrease acrylamide formation in cooked foods" (the "Experiment"). The Experiment was conducted at the Winton Hill Business Center, a P&G facility in Cincinnati, Ohio.

9. I recorded the details of how I conducted the Experiment on pages 2 and 3 in my P&G Lab Notebook #WHS 2688.

10. A true and correct copy of the cover, instruction sheet, and pages 2 and 3 of my P&G Lab Notebook #WHS 2688 I attached hereto as Exhibit A. The dates on Exhibit A have been blacked out, but all of the dates are before September 10, 2002.

11. In the Experiment's first step, baking potatoes were boiled for two hours. The potatoes were then peeled and mashed with a fork.

12. Next, 100 grams of the mashed potatoes that were prepared in the Experiment's first step were mixed with 100 grams of distilled and de-ionized water, and the resulting mixture was homogenized until it was uniform and no lumps were visible.

13. Next, four samples were prepared. Each sample consisted of 30 grams of the mixture described above in paragraph 12, mixed with 30 grams of distilled and de-ionized water. Each sample was placed in an eight ounce glass jar, and the four samples were labeled A1, A2, E1 and E2, respectively.

14. A solution was prepared consisting of 500 units of asparaginase dissolved in 1.0 milliliter of distilled and de-ionized water. One unit of asparaginase is defined as the amount of asparaginase that will liberate 1.0 micromole of NH_3 from L-asparagine per minute at 37° C and a pH of 8.6. The asparaginase I used was ordered before September 10, 2002, from VWR, a vendor that arranges ordering and shipping of scientific products within P&G. A true and correct copy of the email I sent to VWR asking that they order the asparaginase from Sigma-Aldrich Inc. is attached hereto as Exhibit B. A true and correct copy of the Sigma-Aldrich Inc. invoice for the asparaginase order is attached hereto as Exhibit C. The dates on Exhibits B and C have been blacked out, but all of the dates are before September 10, 2002.

15. 100 microliters of the asparaginase solution described above in paragraph 14 was added to the jar labeled E1, and 100 microliters of the same solution was added to the jar labeled E2. No asparaginase solution was added to the jars labeled A1 and A2, as those jars served as controls.

16. Next, the four samples described above in paragraph 15 were allowed to stand at room temperature for 30 minutes with occasional stirring to allow the asparaginase in the jars labeled E1 and E2 to react with the asparagine in the potatoes.

17. The four samples described above in paragraph 16 were then micro-waved for two minutes to deactivate the asparaginase in the jars labeled E1 and E2.

18. The four samples described above in paragraph 17 were then micro-waved in two minute sessions until the samples were cooked. This required four two minute sessions, for a total of eight minutes.

19. The four samples described above in paragraph 18 were then sent to P&G's Foods and Beverages Analytical/Microbiology lab for analyses of the acrylamide, asparagine and aspartic acid contents of the samples. Deborah K. Ewald performed the acrylamide testing, and Janice N. Batchelor performed the asparagine and aspartic acid testing.

20. I received the results of the acrylamide analysis from Deborah Ewald. These results were tabulated in a spreadsheet, a true and correct copy of which is attached hereto as Exhibit D. I also recorded these results on page 3 of my Lab Notebook #WHS 2688 (Exhibit A). The dates on Exhibit D have been blacked out, but all of the dates are before September 10, 2002.

21. The lab results show that for the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the acrylamide levels were 21,605 and 20,543 parts per billion ("ppb") respectively. For the jars labeled E1 and E2 (the two samples that were treated with the asparaginase solution), the acrylamide levels were 385 and 164 ppb, respectively.

22. The results described above in paragraphs 20 and 21 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the acrylamide levels to be reduced by over 98% after cooking, as compared to the levels of acrylamide in the untreated samples in the jars labeled A1 and A2.

23. I received the results of the asparagine and aspartic acid analyses from Janice N. Batchelor. Those analyses were performed before September 10, 2002. A true and correct copy of those results is attached hereto as Exhibit E. I also recorded those results on page 3 of Lab Notebook #WHS 2688 (Exhibit A).

24. The lab results I received show that for the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the asparagine levels were 1131.0 and 1041.6 parts per million ("ppm"), respectively. For the jars

labeled E1 and E2 (the two samples that were treated with asparaginase solution), the asparagine levels were 129.5 and 195.5 ppm, respectively. For the jars labeled A1 and A2 (the two samples that were not treated with the asparaginase solution), the aspartic acid levels were 189.2 and 178 ppm, respectively. For the jars labeled E1 and E2 (the two samples that were treated with the asparaginase solution), the aspartic acid levels were 1307 and 1826.5 ppm, respectively.

25. The results described above in paragraphs 23 and 24 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the asparagine levels to be reduced by over 85% after cooking, as compared to the levels of asparagine in the untreated samples in the jars labeled A1 and A2.

26. The results described above in paragraphs 23 and 24 demonstrate that, in the case of the samples in the jars labeled E1 and E2, the addition of asparaginase to the mashed potato mixture caused the aspartic acid levels to be increased by over 753% after cooking, as compared to the levels of aspartic acid in the untreated samples in the jars labeled A1 and A2.

27. I explained the Experiment, its results, and the significance of the results, to Dr. Kwan Y. Lee, a Principal Scientist in P&G's Food and Beverages Analytical/Microbiology Division in Cincinnati, Ohio. I also showed him pages 2 and 3 of my P&G Lab Notebook #WHS 2688 (Exhibit A). Dr. Lee signed and dated page 2 of my entry in Lab Notebook #WHS 2688. He also dated page 3, but did not sign it. I believe that Dr. Lee's failure to sign page 3 was an oversight. The dates on Exhibit A have been blacked out, but all of the dates are before September 10, 2002.

28. I understand that claim 1 of the Zyzak '137 application reads as follows:

> A method for reducing the level of asparagine in a food material, comprising adding an asparagine-reducing enzyme to the food material before heating.

29. The Experiment discussed above in paragraphs 11 through 19 corresponds to claim 1 of the Zyzak '137 application. In the Experiment, I reduced the level of asparagine in mashed potatoes (a food material) by adding asparaginase (an

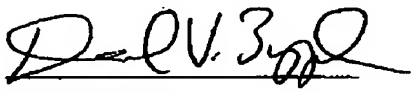
asparagine-reducing enzyme) to a mixture of mashed potatoes and water before I heated the mixture in a microwave oven.

30. I understand that claim 10 of the Zyzak '137 application reads as follows:

> A method for reducing the level of acrylamide in food, comprising:
>
> 1) adding an asparagine-reducing enzyme to a food material, wherein said food material comprises asparagine;
>
> 2) optionally mixing the enzyme with the food material;
>
> 3) allowing a sufficient time for the enzyme to react with asparagine;
>
> 4) optionally deactivating or optionally removing the enzyme; and
>
> 5) heating the food material to form the finished food product.

31. The Experiment discussed above in paragraphs 11 through 19 corresponds to claim 10 of the Zyzak '137 application. In the Experiment, I mixed a solution containing asparaginase (an asparagine-reducing enzyme) with a mixture of mashed potatoes (a food material that contains asparagine) and water. I then allowed the mixture of asparaginase solution, mashed potatoes and water to sit for 30 minutes, which was sufficient time for the asparaginase to react with asparagine. I then deactivated the asparaginase by micro-waving the mixture for two minutes. I then heated the mixture for a total of eight minutes in a micro-wave oven, at which point it was cooked. I then had the cooked material tested for acrylamide. The acrylamide levels were more than 98% lower than acrylamide levels in the control runs that were not treated with asparaginase solution. Immediately after completing the experiments discussed in paragraphs 11 through 19, and receiving the results discussed in paragraphs 20 through 24, I worked diligently with my co-inventors and a patent attorney employed by the Procter & Gamble Company, to further reduce the present invention to practice and to prepare and file U.S. patent application Serial No. 10/606,137.

32. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the Zyzak '137 application or any patent issuing therefrom.



David V. Zyzak

November 28, 2006

Dated

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED
PURSUANT TO 37 C.F.R. § 1.131
Application No. 10/606,137
P&G Docket No. 9043MXL

EXHIBIT A

LABORATORY BOOK NO. WHS2688 CORRESPONDING LOOSE-LEAF NOTEBOOK DATE ISSUED [redacted]

ASSIGNED TO David Zyzak DATE OF LAST ENTRY

DATE RETURNED

TRANSFERRED TO: DIVISION 5155

NAME NAME NAME

DATE DATE DATE WHTC

INSTRUCTIONS FOR ENTERING DATA IN LABORATORY NOTEBOOKS

LABORATORY NOTEBOOKS ARE LEGAL DOCUMENTS. NOTEBOOKS NOT COMPLYING WITH SPECIFICATIONS MAY BE RETURNED FOR CORRECTION.

DATA ENTRIES

A. Enter data into the notebook as the work is being performed. Entries must be made in permanent black ink only. DO NOT USE PENCIL OR FELT TIP PEN to enter data in notebook. Enter the date the work is started at the top of the page. Enter the title of the work on the top line immediately following the date.

B. Describe the purpose of the work at the outset.

C. Give a narrative description of what was done, and indicate the sequence in which each step was taken. Cross-reference data entries as appropriate for maximum clarity. For example, if analytical results on coded samples are entered in the notebook, enter the notebook and page number where the sample description can be found and provide references to procedures or analytical methods used.

D. Define trade-named materials, acronyms or jargon, the first time they are used. Show the mathematical formula for all calculations and a sample calculation if the principle is not obvious. Computer programs used for data analysis should be referenced.

E. Enter factual results only. These include data as well as observations. Opinions should not be recorded in the notebook. Comments implying failure should be avoided.

F. Make entries on a given subject on consecutive pages where practical. Restrict each page to a single subject or test. When considerable work on a single subject is to be done, reserve a single notebook for the work whenever practical.

G. Do not skip pages. When unavoidable, cross through blank page(s) in ink, initial and date. Blank partial pages should also be crossed through in ink, initialed and dated.

H. Do not erase or use correction fluid in notebooks. When corrections are necessary:
   1. Cross out the original entry such that it remains legible;
   2. Enter the correction along with an explanation as to why the correction is necessary; and
   3. Date and initial the correction.

I. DO NOT USE HIGHLIGHTER

ATTACHMENTS

A. Limit attachments to no more than one item every other page. Use rub-on glue or tape only to make attachments. Place tape or glue on at least three entire edges of the item being attached. DO NOT STAPLE attachments in notebook.

B. Attachments must be placed BETWEEN the DOUBLE LINES on the top and bottom of a page. Sign and date the item across the point of attachment. Do not reduce attachment unless the full size original exists in a referenced loose-leaf notebook. The reduction must be completely legible.

C. FOLD-OUT ATTACHMENTS AND OVERLAPPING ATTACHMENTS ARE STRICTLY PROHIBITED.

SIGNATURES AND DATES

A. Minimum patentability standards require each notebook page to have two signatures: the person doing the work and a corroborating witness. A corroborating witness must be an unbiased non-inventor who preferably witnessed performance of the work in its entirety. The person doing the work must sign and date each notebook page.

B. Good Laboratory Practices (GLP's) require all entries on a page that are made on a date other than the date at the top of the page to show the current date and initials of the person making the entry.

C. Good Manufacturing Practices (GMP's) require production records to be signed by the person doing the work and by an independent observer. Laboratory Control records are required to be dated and signed by the person doing the work and by the person reviewing the records.

RESPONSIBILITY

A. The person to whom this book is issued is responsible for returning it to the issuing Library as soon as it is no longer in active use.

B. Incomplete notebooks can be transferred to another person if both parties agree to the transfer and certify the transfer with the Internal Records Administrator at the issuing Library.

C. This notebook must be indexed and have keywords assigned by the user before returning it to the Library. It must also include explicit cross-reference to all other loose-leaf and hardbound notebooks which contain related work.

THIS BOOK IS THE PROPERTY OF THE PROCTER & GAMBLE COMPANY [redacted]

[redacted]

2

Date [redacted] P&G Restricted

Subject Use of Asparaginase to decrease acrylamide formation in cooked foods

Background: Our data suggest that asparagine is the source of acrylamide formation in heated potatoes (and possibly in all foods). If we use the enzyme asparaginase, which converts asparagine to aspartic acid, we should be able to decrease acrylamide formation in heated potatoes.

Reagents / Supplies:

① Mashed potatoes - made by boiling baking potatoes, obtained from local supermarket, for 2 hrs. The boiled potatoes are de-peeled and mashed with a fork.

② Asparaginase
Sigma A 2925 (500 units) dissolved in 1.0 mL distilled and deionized water.
One unit definition: One unit will liberate 1.0 μmole of NH_3 from L-asparagine per minute at pH 8.6 at 37°C
[Vial is labeled as 3.6 mg solid and protein content is 40%]

③ Panasonic Microwave Model NN-S548A

Procedure to prepare mashed potato slurry:
① Take 100 g of mashed potatoes
② Add 100 g of distilled and deionized water
③ Homogenize until uniform and no lumps are visible

Experiments:
① Take 30 g of mashed potato slurry and place in 8oz glass jar.
② Add 30 g distilled and deionized water.

This was done to prepare 4 jars labeled A1, A2, E1 + E2

③ To jars labeled E1 and E2, add 100 μL of the asparaginase solution. This is equivalent to 50 units or approximately 1.44 mg protein.

Worker's Signature [redacted] Date [redacted]
Corroborating Witness [redacted] Date [redacted]

Date [redacted] P&G Restricted

Subject Asparaginase continued from p. 2

④ Let samples stand at room temperature for 30 min with occassional stirring/swirling every 5 min.

⑤ To deactivate enzyme: Microwave samples for 2 min on high setting. Treat samples without asparaginase (A1 + A2) the same. (E1 + E2)

[Microwaving was done in pairs A1 & A2 together; E1 + E2 together]

⑥ Continue to microwave in 2 min sessions until slurry is dried. This took 4 sessions and all 4 samples (A1, A2, E1, E2) turned reddish-brown. There was no apparent difference between A1, A2, E1, and E2 in color or degree of dryness. The microwave drying appeared to work well. The aroma of A1, A2, E1, + E2 were very similar - vegetable protein like, similar to a mild Hydrolyzed Plant Protein (HPP) with potato undertones.

⑦ Submit samples for acrylamide analysis and asparagine analysis.

(Maxxam Labs)

Sample	Acrylamide (ppb)	Asparagine (ppm)	Aspartic Acid (ppm)
A1	21,605	1131.0	189.2
A2	20,543	1041.6	178.0
E1	385	129.5	1307.0
E2	164	195.5	1826.5

Results:

① 99.7% inhibition of acrylamide formation with asparaginase.

Worker's Signature [redacted] Date [redacted]

Corroborating Witness [redacted] Date [redacted]

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED
PURSUANT TO 37 C.F.R. § 1.131
Application No. 10/606,137
P&G Docket No. 9043MXL

EXHIBIT B

[redacted] David Zyzak-DV To: Special Wwr-IM/PGI
[redacted] 11:06 AM cc:
Subject: order

I would like to order the following chemical from sigma 1-800-325-3010.

Item	catalog Number	quantity	size	price
Asparaginase	A 2925	3	500 units	82.35 (each)

needed by Tuesday[redacted]

Please mail to:

Debbie Ewald (Room F1B30)
P&G
6071 Center Hill Ave.
Cincinnati, OH 45224

Please charge to my AMEX:
3787 325587 41009
[redacted]

Thanks,
David Zyzak

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED
PURSUANT TO 37 C.F.R. § 1.131
Application No. 10/606,137
P&G Docket No. 9043MXL

EXHIBIT C

SOLD TO:
PROCTER & GAMBLE CO (SELECT)
PO BOX 5555
CINCINNATI OH 45201-5555

SHIP-TO:
[illegible]
PROCTER & GAMBLE CO
ROOM [illegible]
[illegible]
6071 CENTER HILL AVE
CINCINNATI OH 45224

BILL TO:
PROCTER & GAMBLE CO (SELECT)
PO BOX 5555
CINCINNATI OH 45201-5555

SIGMA-ALDRICH

REMITTANCES TO: SIGMA-ALDRICH INC., PO BOX 932594
ATLANTA, GA 31193
fein: 43-17422718
FOR GENERAL INFORMATION
Sigma - 800-521-8956 Supelco - 800-247-6628
Aldrich, Fluka, Riedel-de-Haen - 800-771-6737
e-mail-sigald@sial.com Home Page: http://www.sigma-aldrich.com
Phone Collect from Anywhere in the world (314)771-5750

INVOICE NUMBER
98541317
SHIP DATE/INVOICE DATE
[redacted]
TERMS/DUE DATE
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SOLD TO CUSTOMER NUMBER
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CONTACT: 513-[illegible]-1100

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"To ensure proper postings of your payments, please indicate invoice number on your payment advice & mail it to the remittance address indicated. Thank You."

All sales are expressly limited to the conditions upon the terms and conditions appearing on the front and back of this form.

Total Amount Due	FCA 0.0[illegible]
Currency	[illegible]
Page	1 / 1

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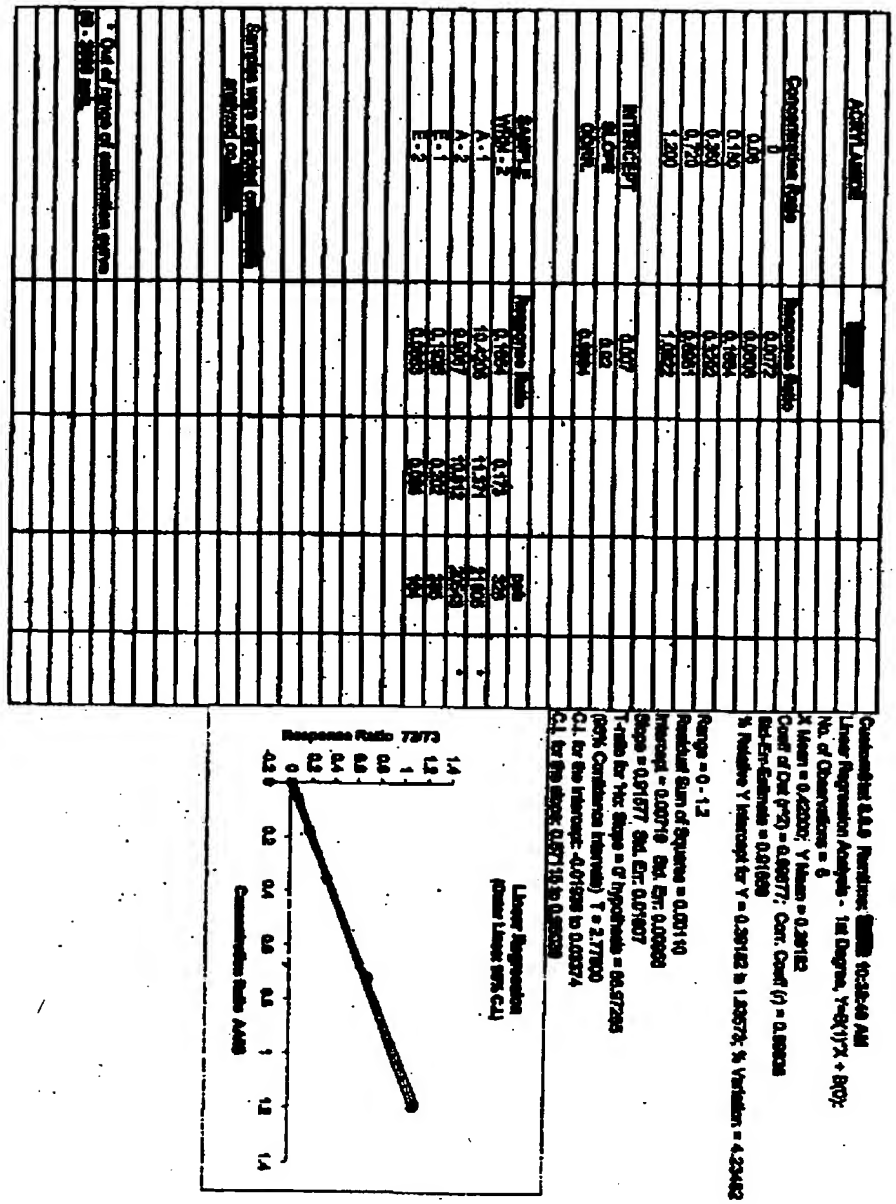
We are Committed to the Success of our Customers through Science, Technology and Service.

Manager

JHOLMES - 07/18/2006

DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED
PURSUANT TO 37 C.F.R. § 1.131
Application No. 10/606,137
P&G Docket No. 9043MXL

EXHIBIT D



DECLARATION OF DAVID VINCENT ZYZAK SUBMITTED
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Application No. 10/606,137
P&G Docket No. 9043MXL

EXHIBIT E

CLASS-VP V 5.03 External Standard Report Page 1 of 1 (6)

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Data Name: C:\CLASS-VP\DATA\SOREN[redacted]
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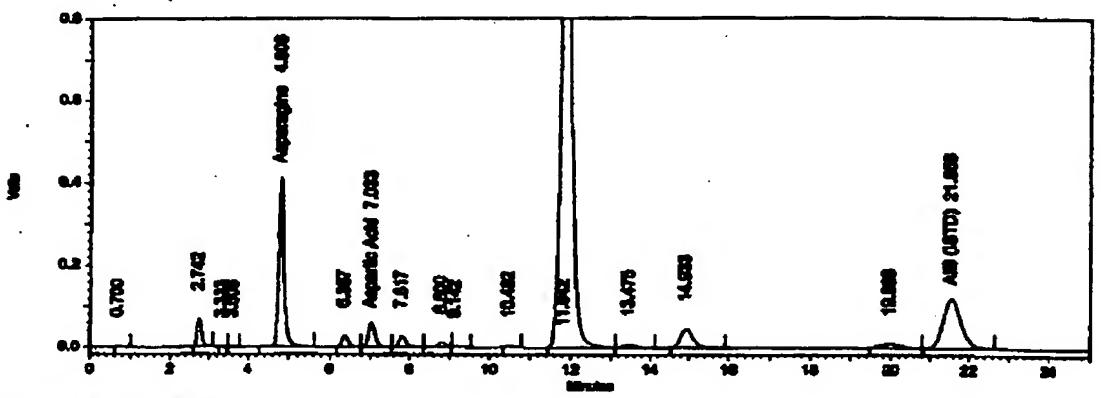

Sample Amount: 1

Multiplier Factor: 1

Fluorescence Detector (Ex:260nm, Em:313nm)

Pk #	Name	Retention Time	Area	ISTD concentration	Units
5	Asparagine	4.81	3949539	1131.042	ppm
7	Aspartic Acid	7.03	696440	189.169	ppm
16	AIB (ISTD)	21.68	3765554	0.000	ppm

000148

CLASS-VP V 5.03 External Standard Report Page 1 of 1 (7)

Method Name: C:\CLASS-VP\METHODS\Asparagine extended.met
Sequence Name: C:\CLASS-VP\SEQUENCE\GLUCOSAMINE\[redacted].seq
Data Name: C:\CLASS-VP\DATA\SOREEN\[redacted]
Sample ID; A2 | 4th Sample Set
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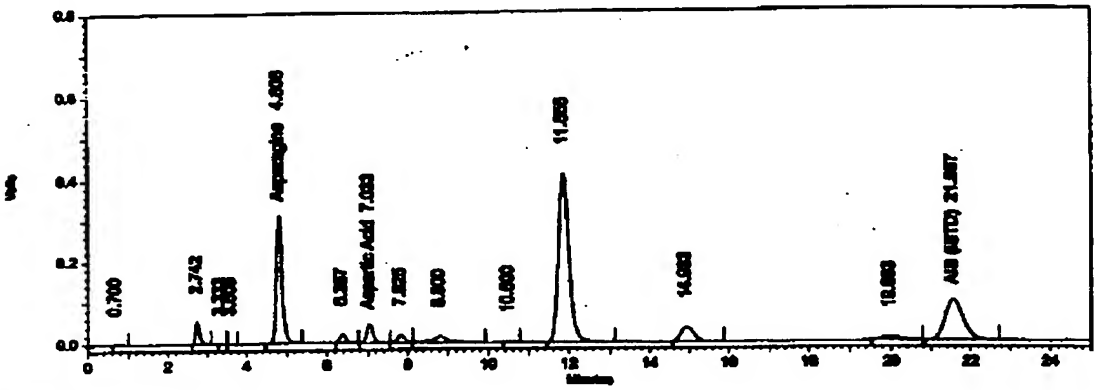


Sample Amount: 1

Multiplier Factor: 1

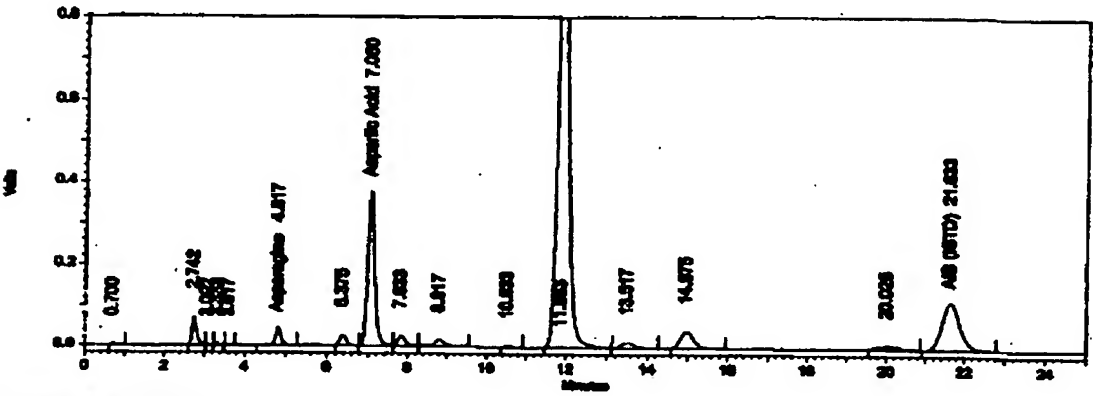
Fluorescence Detector (Ex:260nm, Em:313nm)

Pk #	Name	Retention Time	Area	ISTD concentration	Units
5	Asparagine	4.81	2949846	1041.552	ppm
7	Aspartic Acid	7.03	532709	177.953	ppm
14	AIB (ISTD)	21.57	3052920	0.000	ppm

000149

[redacted] CLASS-VP V 5.63 External Standard Report Page 1 of 1 (5)

[redacted]thod Name: C:\CLASS-VP\METHODS\Asparagine extended.met
[redacted]quence Name: C:\CLASS-VP\SEQUENCE\GLUCOSAMINE[redacted].seq
[redacted]Data Name: C:\CLASS-VP\DATA\SOREEN[redacted]
Sample ID: E1 1st Sample Set - Asparagine - Hydrolyzed
User: System
Acquired: [redacted] 7:00:42 PM
[redacted]ished: [redacted] 7:27:20 PM



Sample Amount: 1

Multiplier Factor: 1

Fluorescence Detector (Ex:260nm, Em:313nm)

Pk #	Name	Retention Time	Area	ISTD concentration	Units
6	Asparagine	4.82	461708	129.529	ppm
8	Aspartic Acid	7.05	4562875	1307.031	ppm
16	AIB (ISTD)	21.63	3717360	0.000	ppm

000150

CLASS-VP V 5.03 External Standard Report Page 1 of 1 (9)

Method Name: C:\CLASS-VP\METHODS\Asparagine extended.met
Sequence Name: C:\CLASS-VP\SEQUENCE\GLUCOSAMINE[redacted].seq
Data Name: C:\CLASS-VP\DATA\SORBEN[redacted]
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User: System
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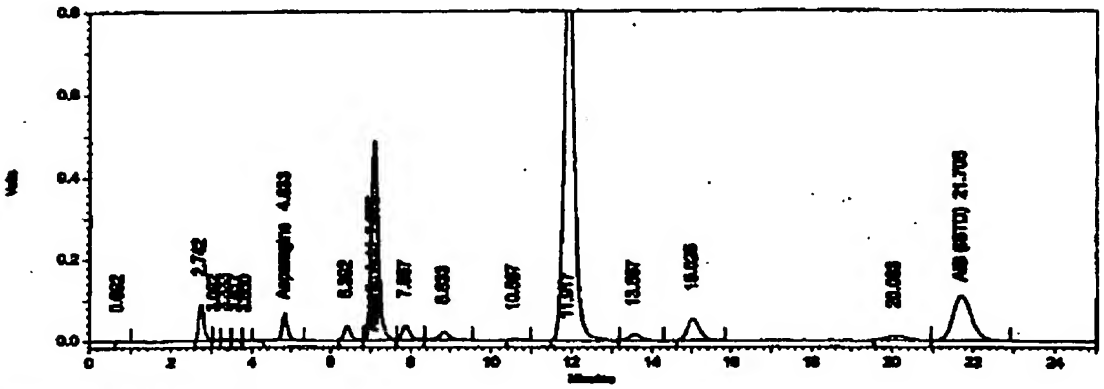

Sample Amount: 1

Multiplier Factor: 1

Fluorescence Detector (Ex:280nm, Em:313nm)

Pk #	Name	Retention Time	Area	ISTD concentration	Units
7	Asparagine	4.83	641523	195.516	ppm
9	Aspartic Acid	7.06	5932060	1826.512	ppm
17	AIB (ISTD)	21.71	3465326	0.000	ppm

000151